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07 April 2005

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Prevention of diabetic retinopathy by inhibition of the visual cycle

All patent and non-patent references cited in the application are also hereby incorporated by reference in their entirety.

Field of Invention

The present invention relates to compounds capable of inhibiting the visual cycle and/or dark adaptation, such as especially retinoids, and their use for treatment and prevention of non-degenerative retinal diseases with specific emphasis on the prevention and treatment of diabetic retinopathy, branch retinal vein occlusion, central retinal vein occlusion, open-angle glaucoma, neovascular glaucoma, and other diseases of the retinal and/or optic nerve where an imbalance between metabolic demand and blood supply contribute to the development of tissue damage.

Background of invention

Patients suffering from diabetes occasionally develop diabetic retinopathy, the leading cause of blindness of people ages 20-60.

Diabetic retinopathy is a non-degenerative disease of the small blood vessels of the retina, which is the light-sensitive tissue in the back of the eye. Diabetic retinopathy is related to the abnormally elevated levels of blood sugar in diabetes, and the retinal changes include impaired vascular function, vascular leakage, vascular congestion, vascular occlusion, tissue swelling (edema) and tissue ischemia. Metabolic hyperactivity and hyperfusion are also implicated in the development of diabetic retinopathy.

The lower grades of diabetic retinopathy are collectively called diabetic background retinopathy or nonproliferative diabetic retinopathy. Leakage of fluid from diseased retinal vessels may cause swelling of the center of the retina (the foeva, which is in the center of the macula) and hence cause blurred vision and severe visual loss

secondary to diabetic macular edema. Reduced retinal perfusion secondary to microvascular occlusion may cause the growth of new vessels from intact vessel. Such neovascularizations (proliferative diabetic retinopathy) may cause preretinal hemorrhage, traction detachment of the retina, and severe visual loss. About half of the people with proliferative retinopathy also experience macular edema, which can occur at any stage of diabetic retinopathy.

The conventional primary means of treating diabetic retinopathy target its macular edema and proliferative retinopathy stages. The treatment consists of producing multiple circumscribed photocoagulation lesions of the outer layers of the retina, using, for instance, blue-green 514.5 nm light from an argon ion laser. Such lesions induce focal necrosis and permanent functional loss, but if applied properly, the treatment may result in improved preservation of some visual function rather than complete or incapacitating visual loss. The function of the center of the visual field is given special priority. The mechanism of action of photocoagulation treatment involved reduction of oxygen demand by removal of a large proportion of the retinal photoreceptors and enhanced drainage of fluid from the retina to the choroid, and probably also perfusion reduction.

If severe preretinal bleeding or traction from fibrotic proliferations occur, surgical removal of blood, fibrous tissue, and vitreous gel can be performed. Vitrectomy is usually accompanied by retinal photocoagulation treatment if this has not been completed on beforehand. Overall, photocoagulation and vitrectomy are succesful only in reducing the rate of visual loss in patients with diabetic retinopathy to about half of the spontaneous rate. Photocoagulation has considerable drawbacks because it is only moderately effective and because it invariably induces loss of vision corresponding to the location of the coagulation injury.

Once a patient has been diagnosed with diabetic retinopathy the risk of bleeding will always be present and repeated treatment may be needed. Diabetic retinopathy has no early warning signs and macular edema and proliferative diabetic retinopathy can develop without any premonitory symptoms, therefore diabetic retinopathy may develop undetected to the severe stages of the disease.

Currently, besides attempting to control levels of blood sugar, blood pressure and blood cholesterol no method for prevention of diabetic retinopathy is known. A preventive mode of treatment would substantially reduce occurrence of eyesight loss in diabetic patients and ease the course of the disease. Furthermore, a modality of treatment that is better than conventional treatment or an effective adjunct to conventional treatment will be of considerable benefit to patients with diabetes.

Retinoids are a class of compounds with several functional activities consisting of four isoprenoid units joined in a head-to-tail manner (9). Several such compounds are vitamins or provitamins because they possess the biological activity of vitamin A which is not synthesized in the body and must be derived from the diet. Retinoids are also hormones with intracrine activity and capable of binding to nuclear receptors resulting in the alteration of cell division and immune function.

The visual response in vertebrates begins by a light-induced isomerization of the rhodopsin chromophore, 11-*cis*-retinal, in the photoreceptor cells of the retina. Light bleaches 11-*cis*-retinal to *all-trans*-retinol (vitamin A), which cannot be synthesized de novo by mammals. The bleaching of the purple-red rhodopsin to visual yellow initiates retinal visual signaling.

The recovery mechanism from bleach requires reconversion of the chromophore to 11-*cis*-retinal by a multiple of enzymatic reactions called the visual cycle. This process takes place in the retinal pigment epithelium (RPE), a cell layer lying adjacent to the photoreceptor cells (1).

The color and sensitivity to light of the rhodopsin protein in the photoreceptors depend upon the presence of 11-*cis*-retinal. Disruption of the visual cycle retards restoration of the visual function after exposure to bright light. Notably dark adaptation and night vision are deficient in subjects with deficient uptake of vitamin A (2). Night blindness can also be induced by dietary substitution of vitamin A with retinoic acid in rats (3).

The retinoids comprise a group of natural and synthetic compounds with structural similarities and affinity for biological receptors for vitamin A (retinol). Retinoids possess dual functional activities as hormones and vitamins, respectively. They

stimulate nuclear retinoid receptors controlling cell division and immune function, and they absorb photons in the retina and then initiate the visual (vitamin A) cycle.

A synthetic analogue of vitamin A, the retinoid isotretinoin (13-*cis*-retinoic acid), is commonly used for treatment of severe nodular acne and various other skin disorders for almost two decades. Known side-effects of isotretinoin treatment are night blindness and excessive glare sensitivity (4-6) and experiments have shown that isotretinoin exerts its effect by inhibiting the processing of vitamin A in the retina and the RPE (7-8).

Other retinoids, such as the 11-*cis*-retinoids have been shown to inhibit enzymes involved in catalyzing processes of the visual cycle and thereby slow dark adaptation in treated subjects (13).

During dark adaptation the photoreceptor layer removes considerable amounts of oxygen from the inner retina leading to an unusually low oxygen tension. Retinal hypoxia has been shown to play a major role in the development of diabetic retinopathy and elimination of periods with full dark adaptation by low levels of background light at night has been suggested as a therapeutic against diabetic retinopathy (14).

Summary of Invention

Administration of compounds capable of inhibiting the visual cycle and/or dark adaptation, including retinoids, provides a novel method for prevention and treatment of diabetic retinopathy. This principle also applies to other ischemic and/or hypoxic diseases of the retina and the optic nerve, the latter consisting of nerve cell axons that are extensions from the ganglion cell bodies of the retina. Such conditions include branch retinal vein occlusion, central retinal vein occlusion, open-angle glaucoma, and neovascular glaucoma.

The present invention, thus, has the potential to eradicate the most predominant causes of blindness of people of the working ages in industrialized countries and substantially reduce the sufferings of patients with diabetes.

The use of compounds capable of inhibiting the visual cycle and/or dark adaptation, such as retinoids effective of inhibiting the visual cycle, in pharmaceutical compositions and methods for prevention and treatment of non-degenerative retinal disorders are described herein.

The present invention is in one aspect directed to the use of at least one compound capable of inhibiting the visual cycle and/or dark adaptation, such as at least one retinoid effective of inhibiting the visual cycle and/or dark adaptation in an individual, in the manufacture of a medicament for prevention or treatment of a non-degenerative retinal disorder, or associated symptoms and complications thereof in a mammalian, including a human.

In a further aspect the present invention is directed to a medicament for prevention or treatment of a non-degenerative retinal disorder comprising at least one compound capable of inhibiting the visual cycle and/or dark adaptation, such as at least one retinoid effective of inhibiting the visual cycle in an individual, as an active ingredient.

In another aspect, the present invention comprises a method for prevention or treatment of a non-degenerative retinal disorder, or associated symptoms and complications thereof, in a mammalian, including a human, comprising administering to said mammalian, including a human, a pharmaceutically efficient amount of at least one compound capable of inhibiting the visual cycle and/or dark adaptation, such as at least one retinoid effective of inhibiting the visual cycle in an individual.

In yet another aspect there is provided a pharmaceutical composition comprising at least one compound capable of inhibiting the visual cycle and/or dark adaptation, or at least one retinoid, preferably effective of inhibiting the visual cycle, wherein said pharmaceutical composition is suitable for intravitreal implantation.

Definitions

The terms "treatment" and "treating" as used herein refer to any treatment of a disease in a mammalian, particularly a human, and generally include inhibiting the

disease, i.e., arresting its development, or relieving the disease, i.e., causing regression of the disease. Treating also refers to providing a beneficial alteration in one or more of the symptoms of a disease state or reducing or eliminating the disease state itself. It will be appreciated that a beneficial alteration can include transitory or permanent reduction or elimination of the symptom.

The terms "prevention" and "preventing" as used herein refer to prevention of the occurrence of a disease in a subject that may be predisposed to a disease but has not yet been diagnosed as having it. It will also be appreciated that "prevention" and "preventing" can also involve a reduction in the likelihood of adverse consequences of a pathological state. Thus, "prevention" and "preventing" as used herein can also refer to prophylaxis.

For therapeutic purposes the term "a pharmaceutically efficient amount" means the amount of a pharmaceutical agent or multidrug therapeutic which elicits a positive response on at least one symptom of a disease state, or which acts prophylactically to reduce the likelihood of at least one pathological symptoms or consequences of a disease state, i.e., to inhibit the onset or progression of the disease.

The term "administering" in the context of "administering to a mammalian" refers to delivering the therapeutic agents in question to an organism. Administration can be systemic, topical, or local administration as described herein, or the implantation of a slow-release device to the subject.

As used herein, "therapeutic agent" means any agent useful for therapy.

"Inhibition of the visual cycle" or "Inhibiting the visual cycle" as used herein means stopping, eliminating, or slowing down any or more processes of the visual cycle, for example inhibition of the formation of 11-*cis*-retinoids, such as 11-*cis*-retinal, as measured invasively or in organ culture by spectrophobic assays, such as inhibition of the conversion of 11-*cis*-retinal into dark adapted rhodopsin as measured by invasively or in organ culture by spectrographic assays, for example inhibition of one or more enzymes in the visual cycle leading to decrease of dark adapted rhodopsin, such as eliminating the photoisomerization step of the cycle by catalyzing the isomerization of 11-*cis*-retinal to *all-trans*-retinal, or for example by depletion of

stores of 11-*cis*-retinoids. In vivo monitoring of such effects can be made using flicker photometric determination of the spectral absorption of the fundus of the eye or dark adaptometry using the method of Goldmann-Weeker or electroretinographic assessment of scotopic and photopic retinal function.

Description of Drawings

Figure 1. Schematic representation of the visual cycle.

Figure 2. Chemical structures of compounds of the visual cycle.

Figure 3. Schematic representation of the proposed activity of aromatic amines in the visual cycle.

Detailed description of the invention

Compounds capable of inhibiting the visual cycle and thereby slowing down the regeneration of the photopigment of the retina provides useful methods of prevention and treatment of diabetic retinopathy and other non-degenerative disorders of the eye.

The present invention relates to pharmaceutical compositions and methods for the inhibition of the visual cycle comprising such compounds.

The visual pigment rhodopsin in vertebrates contains an 11-*cis*-retinal protonated Schiff base and during visual response a photon absorbed by a rhodopsin molecule causes a *cis* to *trans* photoisomerization of the rhodopsin chromophore 11-*cis*-retinal to *all-trans*-retinal in the outer segment discs of rod photoreceptors (Figure 2). The bleached photopigment activates the signal transduction cascade leading to membrane hyperpolarization and retinal visual signaling.

Recovery from bleach and dark adaptation involves rhodopsin regeneration by the reconversion of the *all-trans*-retinol into 11-*cis*-retinal by enzymatic reactions in

completion of the visual cycle, also called the vitamin A cycle (Figure 1). During the visual cycle *all-trans*-retinal is reduced to *all-trans*-retinol (vitamin A) by *all-trans*-retinol dehydrogenase. The *all-trans*-retinol is released from the outer segment disc and taken up by an underlying RPE cell. Lecithin retinol acyltransferase (LRAT) catalyzes the generation of *all-trans*-retinyl esters from vitamin A and isomerohydrolase processes the esters into 11-*cis*-retinol which is oxidized by 11-*cis*-retinol dehydrogenase to form 11-*cis*-retinal chromophore.

In several animal models suffering from retinal disorders light accelerates retinal degeneration and dark rearing has been shown to prolong rod cell survival. Furthermore, slowing of rhodopsin regeneration or the absence of rhodopsin in knockout mice confer protection from light damage. The slowing of rhodopsin regeneration by inhibition of the formation of 11-*cis*-retinal provides a therapeutic strategy for non-degenerative retinal disorders by reducing the number of photoisomerization events.

The protein RPE65 plays an important role in visual cycle function. RPE65 knockout mice are unable to produce substantial levels of 11-*cis*-retinoids and *all-trans*-retinyl esters where found to accumulate in RPE cells of these mice (19). Recovery of visual function after transgenic correction of this defect has demonstrated that chronic inhibition of the visual cycle is possible without confering irreparable damage to the retina (18).

<u>Compounds capable of inhibiting the visual cycle and/or dark adaptation</u>

Examples of compounds capable of slowing the visual cycle and/or dark adaptation include but are not limited to the following compounds.

The vitamin A analogue isotretinoin (13-*cis*-retinoic acid or Accutane) is commonly used to treat severe acne. A side effect of treatment with isotretinoin is reduced night vision because of its inhibitory effect on 11-*cis*-retinol dehydrogenase activity in RPE cells. However this effect is reversible and isotretinoin administration in rodents has shown that, despite delayed dark adaptation as a result of slowed photopigment recovery, photoreceptor cell loss did not occur, indicating that night

blindness is not a result of rod cell death. Visual function was normal in isotretinoin-treated mice after prolonged dark adaptation.

11-*cis*-retinoids, including molecules of the 11-*cis*-retinol and 11-*cis*-retinal series, have been shown to slow the visual cycle by specifically inhibiting isomerohydrolase (13, 17).

DAPP [1,5-bis(*p*-aminophenoxy)pentane] is an example of a well-characterized non-retinoid inhibitor of dark adaptation. DAPP and monofunctional analogues of DAPP, such as *p*-phenitidine, act by impairing formation and storage of all 11-cis-retinoids in the vertebrate eye (20). The major requirements for a group of inhibitors of the visual cycle and/or dark adaptation of the phenitidine-type are suggested to consist of an aromatic amino group that can form a Schiff base with retinal and a moderately hydrophobic tail in para or meta position (21). These diverse compounds have a common ability to catalyze the energetically favored isomerization of 11-*cis*-retinal to *all-trans*-retinal and are proposed to cause a chemical short-circuit of the visual cycle (Fig. 3).

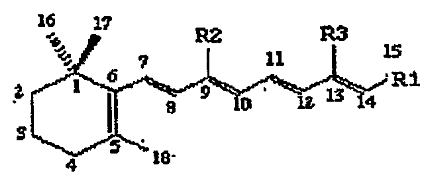
The present invention is directed to use of compounds capable of inhibiting the visual cycle and/or dark adaptation in the prevention and treatment of non-degenerative retinal disorders and in preferred embodiments relates to i) use of compounds capable of inhibiting the visual cycle and/or dark adaptation, especially retinoids effective of inhibiting the visual cycle in an individual, in the manufacture of a medicament for prevention or treatment of a non-degenerative retinal disorder, or associated symptoms and complications thereof, in a mammalian, including a human, ii) a method for prevention or treatment of a non-degenerative retinal disorder, or associated symptoms and complications thereof, in a mammalian, including a human, comprising administration of at least one compound capable of inhibiting the visual cycle and/or dark adaptation, especially at least one retinoid effective of inhibiting the visual cycle, and iii) a pharmaceutical composition suitable for intravitreal implantation comprising at least one compound capable of inhibiting the visual cycle and/or dark adaptation and/or a retinoid, wherein the retinoid preferably is effective of inhibiting the visual cycle.

Retinoids, capable of inhibiting the visual cycle and/or dark adaptation

The term "retinoid" as used herein is directed to all vitamin A derivatives and synthetic molecules displaying vitamin A activity.

The IUPAC-IUB Joint Commission on Biochemical Nomenclature states that "retinoids are a class of compounds consisting of four isoprenoid units joined in a head to tail manner. All retinoids may be formally derived from a monocyclic parent compound containing five carbon-carbon double bonds and a functional group at the terminus of the acyclic portion" (9). The basic retinoid structure is generally subdivided into three segments, namely the polar terminal end, the conjugated side chain, and the cyclohexenyl ring. The basic structures of the most common natural retinoids are called retinol, retinaldehyde, and retinoic acid. Preferred retinoids of this invention are retinoids capable of inhibiting the visual cycle.

In one embodiment the retinoids according to the present invention are illustrated by Formula I:



where R2 and R3 are independent and include CH_3, CH_2OH, CHO, CH_2CH_3, and CF_3. In a particularly preferred embodiment, R2 and R3 are CH_3.

R1 includes CH_2OH, CHO, CO_2H, CH_3, CH_2OCH_3, $CH_2OC_4H_9$, $CH_2OC_6H_5$, $CH_2OC_8H_{17}$, providing *all-trans-* and *cis-* retinyl ethers, R1 includes CH_2OCOCH_3 or $COC_{15}H_{31}$, providing *all-trans-* and *cis-* retinyl esters, R1 includes $CH_2NHCOCH_3$, $CH_2NHCOC_6H_5$, $CH_2NCH_3COCH_3$, $CH_3COC_6H_5$, providing *all-trans-* and *cis-*retinylamine derivatives, R1 includes CH=O, CH=NOH, $CH=NNHCOCH_3$, $CH=C(COCH_2CH_2CH_3)_2$, $CH=C(COCH_2)_2$, $CH=C(COCH_2)_2CH_2CH=C(COCH_2CH_2)_2CH_2$, providing *all-trans-* and *cis-* retinal derivatives, R1 includes COOH, $COOCH_3$, $COOCH_2H_5$, providing *all-trans-* and *cis-*

retinoic acid esters, R1 includes COX where X is an amino acid such as glycine, leucine, phenylalanine, or tyrosine thereby providing an *all-trans-* and *cis-* retinoyl-amino acid, R1 includes $CONHC_2H_5$, $CONHC_3H_7$, $CONH_2—C_2H_4OH$, $CONH_2—C_3H_6OH$, $CONH_3—C_3H_6OH$, $CONHC_6H_5$, $CONH_2—C_6H_4OH$, $CONH_4—C_6H_4OH$, $CONH_2—C_6H_4COOH$, $CONH_4—C_6H_4—COOH$, providing *all-trans-* and *cis-* retinamides. Retinoids thus, include side-chain modified *cis* and multi-*cis* retinoids such as, but not limited to, 13-*cis*-retinoic acid derivatives such as 13-*cis*-retinoic acid, N-ethyl-13-*cis*-retinamide, N-(2-hydroxyethyl)-13 *cis*-retinamide, N-(4-hydroxyphenyl)-13-*cis*-retinamide, N-(13-*cis*-retinoyl) leucine, and N-(13-*cis*-retinoyl) phenylalanine, bifunctional retinoic acid analogues such as 14-carboxy-retinoic acid, ethyl 14-(ethoxycarbonyl) retinoate, and 14-[(ethylamino) carbonyl] -13-*cis*-retinoic acid. Retinoids also include aldehydes, alcohols, or esters of 11-*cis*-retinoid, such as, but not limited to, 11-*cis*-retinol, 11-*cis*-retinal, and 11-*cis*-retinyl bromoacetate. Retinoids also include ring-modified analogues such as the ring-modified *all-trans*-retinoic acid analogues including but not limited to α-retinoic acid, 4-hydroxyretinoic acid, phenyl analogue of retinoic acid, 4-methoxy-2,3,6-trimethyl-phenyl analogue of retinoic acid, 5,6-dihydroretinoic acid, 4-oxoretinoic acid, 3-pyridyl analogue of retinoic acid, dimethylacetylcyclopentenyl analogue of retinoic acid, 2-furyl analogue of retinoic acid, and the 3-thienyl analogue of retinoic acid. Ring-modified retinoids also include retinoid analogues in which the cyclohexenyl ring is replaced by -related structures.

Retinoids also include side-chain modified *all-trans*-retinoic acid analogues such as a C_{15} analogue of retinoic acid, a C_{17} analogue of retinoic acid, a C_{22} analogue of retinoic acid, an aryltriene analogue of retinoic acid, 7,8-dihydroretinoic acid, 8,10-dihydroretinoic acid, 11,12-dihydroretinoic acid. Other side chain modified retinoids include retinol, retinoic acid, and other retinoids with a partially or completely hydrogenated side chain. Still other retinoids having modified side chain include, but are not limited to, retinol or retinoic acid derivatives in which selected double bonds of the side chain are replaced with amide, sulfonamide, or other groups such as, but not limited to, p-(5,6,7,8-tetrahydro-15 5,5,8,8-tetramethyl-2-haphtalene-carbox-amido) benzoic acid.

Other retinoids include both ring- and side-chain-modified analogues of *all-trans*-retinoic acid including, but not limited to (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-

tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid, (E)-4-[2-(5,6,7,8-tetrahydro-8, 8-dimethyl-2-naphthalenyl)-1-propenyl] benzoic acid, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carbamoyl] benzoic acid, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido] benzoic acid, (E)-4-[2-(2,3-dihydro-1,1,2,3,3-pentamethyl-1H-inden-5-yl)-1-propenyl] benzoic acid, 6-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-naphthalenecarboxylic acid, 6-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphtalenyl)-5-methyl-2-naphthalenecarboxylic acid, 6-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-6-benzo [b] thiophenecarboxylic acid, 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl) benzoic acid, and (E)-4-[3-(3,5-Di-tert-butylphenyl)-3-oxo-1-propenyl] benzoic acid.

Detailed descriptions of these and other retinoids can be found (10-12). Other preferred retinoids include glucuronic acid, retinyl β-glucuronide, and retinoyl β-glucuronide.

A large number of retinoids are commercially available (e.g. from Sigma-Aldrich Co., St. Louis, Mo., USA or from F. Hoffmann-La Roche Ltd., Basel, Switzerland, etc.).

In an important embodiment of the present invention it should be appreciated that retinoids capable of inhibiting the visual cycle do not include *all-trans*-retinol (vitamin A), a substrate for LRAT in the visual cycle.

In preferred embodiments of the present invention the medicament for preventing or treating a non-degenerative retinal disorder and/or the pharmaceutical composition suitable for intravitreal implantation comprise(s) at least one retinoid as an active therapeutic ingredient.

Retinoids of the present invention include all known retinoids effective of inhibiting the visual cycle and/or dark adaptation in a subject. In one preferred embodiment of the present invention the at least one retinoid is effective of specifically inhibiting at least one enzyme of the visual (vitamin A) cycle and thereby slowing down the formation of 11-*cis*-retinal. In more preferred embodiments of the present invention the at least one retinoid is capable of inhibiting isomerohydrolase, *all-trans*-retinol dehydrogenase and/or lecithin retinol acyltransferase (LRAT).

In preferred embodiments of the present invention the at least one retinoid is effective of inhibiting the visual cycle in a subject resulting in an inhibition of dark adaptation in said subject as determined by conventional methods as described herein.

In preferred embodiments of the present invention the at least one retinoid is selected from a group consisting of isotretinoin (13-*cis*-retinoic acid), 11-*cis*-retinol, 11-*cis*-retinal, 11-*cis*-retinyl bromoacetate, acitretin, adapalene, bexarotene, etretinate, fenretinide, 4-oxo-isotretinoin, tazarotene, motretinid, arotinoid ethyl ester (Ro 13-6298), arotinoid-free carboxylic acid, arotinoid ethyl sulfone (etarotene, Ro 15-1570), retinaldehyde, *all-trans*-retinyl bromoacetate, *all-trans*-retinyl chloroacetate, and retinoyl beta-glucoronide.

In a preferred embodiment of the present invention the at least one retinoid comprises at least one 11-*cis*-retinoid, such as 11-*cis*-retinol, 11-*cis*-retinal, and 11-*cis*-retinyl bromoacetate, and/or at least one 13-*cis*-retinoid, such as 13-*cis*-retinoic acid. In another preferred embodiment of the present invention the at least one retinoid comprises isotretinoin (13-*cis*-retinoic acid).

In a very preferred embodiment of the present invention the at least one retinoid is selected from the group comprising isotretinoin (13-*cis*-retinoic acid), fenretinide and etretinate.

In specific embodiments of the present invention said at least one retinoid is <u>not</u> *all-trans*-retinoic acid and/or said at least one retinoid is <u>not</u> 9-*cis*-retinoic acid, i.e. the at least one retinoid is any of the above-mentioned retinoids except *all-trans*-retinoic acid and/or 9-*cis*-retinoic acid.

A preferred embodiment of the present invention thus comprises use of at least one retinoid in the manufacture of a medicament for prevention or treatment of a non-degenerative retinal disorder, or associated symptoms and complications thereof in a mammalian, including a human, with the proviso that said at least one retinoid does not comprise *all-trans*-retinoic acid and/or 9-*cis*-retinoic acid.

In one aspect of the invention, a method for treating and/or preventing diabetic retinopathy, or associated symptoms or complications thereof, in a mammalian, including a human, is provided.

Advantageously, in one embodiment the method includes the identification of a subject afflicted with diabetes, and the administration of a pharmaceutically acceptable solution containing a pharmaceutically efficient amount of at least one compound capable of inhibiting the visual cycle and/or dark adaptation, such as a retinoid effective of inhibiting the visual cycle.

Pharmaceutical compositions

Pharmaceutical compositions or medicaments of the present invention comprising compounds capable of inhibiting the visual cycle and/or dark adaptation, include all compositions wherein at least one pharmaceutical compound or composition is contained in an amount effective to achieve its intended purpose.

In addition, pharmaceutical compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically.

Pharmaceutically acceptable carriers may comprise physiologically active compounds that act, for example, to stabilize the composition, and/or to increase or decrease the absorption of the agent. Physiologically acceptable compounds may include, for example, carbohydrates, such as glucose, sucrose, or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low and/or high molecular weight proteins, compositions that reduce the clearance or hydrolysis of the at least one retinoid, or excipients or other stabilizers and/or buffers. Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives which are particularly useful for preventing the growth or action of microorganisms.

Because most retinoids are lipid soluble the use of solubilizers and/or emulsifiers is often desired to produce acqueous retinoid solutions or emulsions. Such solubilizers and emulsifiers are well known to those of skill in the art.

Another preferred embodiment of the present invention include pharmaceutical compositions or medicaments suitable for intravitreal implantation comprising at least one compound capable of inhibiting the visual cycle and/or dark adaptation. Other preferred embodiments include pharmaceutical compositions or medicaments suitable for intravitreal implantation comprising at least one retinoid, preferably a retinoid effective of inhibiting the visual cycle.

Administration

The compounds and/or compositions of the present invention are to be administered preferably to mammalian recipients, most preferably to humans.

The route of administration may be systemic (e.g. oral, parenteral), topical (e.g. eye drops), or local, such as by intravitreal, subretinal, or subtenonal injection or infusion. In one preferred embodiment of the invention the route of administration is local by intraocular injection or infusion. In another preferred embodiment the at least one retinoid is in a device formulation held confined by mechanical or physico-chemical effects. In yet another preferred embodiment the at least one retinoid is in a slow-release formulation. However, a person skilled in the art will appreciate that other effective methods of administration are contemplated by the invention.

One very preferred embodiment of the present invention comprises administering by intraocular injection at least one active agent in a slow-release device formulation to a subject afflicted with diabetes.

Dosages and schedules

A pharmaceutically efficient amount of at least one compound capable of inhibiting the visual cycle and/or dark adaptation is employed in treatment or prevention of a subject. The dosages and repetition interval (the timing of retreatment) of the drug, in the development of the drug formulation as well as in clinical practice, can be adjusted on the basis of titration tests known to persons skilled in the art.

In one embodiment of the present invention the pharmaceutically efficient amount of the at least one active agent is determined as an amount efficient to reduce dark adaptation as measured by conventional methods known to the skilled person. Examples of such tests include but is not limited to electrophysiological measurements (electroretinography, primarily scotopic electroretinography), conventional dark adaptometry according to the method of Goldmann-Weekers or a comparable method, and simple standardized stochastic threshold tests that can be applied outside ophthalmological clinics, e.g. in family practice or in the patient's own home, where the patient could, for example, rest in a completely darkened room for a period of standard length, typically one half hour, and then determine by herself or himself whether a standard luminosity object can be seen or not seen, or a combination of these procedures. In the latter case, the luminous standard object, i.e. a very weak lamp, should be invisible when therapeutic action has been achieved, and should become visible when the therapeutic action has tapered and approaches the level where renewed administration of the therapeutic agent is needed. Consequently, the therapeutic level of night vision suppression is defined and monitored by the patient's response, one eye at a time, to a simple noninvasive test.

Additional means of dosage and readministration interval titration include flicker photometry and fundus reflectometry before and after dark adaptation or during the dark adapted stages only. It is understood that the most complete level of dark adaptation which can be achieved by an individual during treatment may be characterized by absence of normal dark adaptation.

Further methods of achieving optimal dosage and readministration interval include determination of changes in retinal blood flow using fundus photographic, angiographic, laser-doppler based or laser-speckle based methods. Fundus photographic methods of estimating retinal blood flow include measurement of changes in retinal vessel diameter and circulation time. Methods of monitoring the effect of treatment include measurement of changes in retinal thickness using optical coherence tomography and visual grading of the level of diabetic retinopathy or other types of retinal disease.

Specific means of estimating the dose required may include determination of a given patient's stores of vitamin A, vitamin A precursors, and vitamin A metabolites such

that patients with low body stores of such vitamin receive less of the therapeutic agent, etc.

It is understood that the dosage of a pharmaceutical compound or composition of the present invention administered in vivo will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the pharmaceutical effect desired. The ranges of effective doses provided herein are not intended to be limiting and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one skilled in the relevant art.

An example of the dosage range of retinoid required to achieve a clinical effect using systemic administration would be 1 mg of isotretinoin per kg body weight per day to 80 mg of isotretinoin per kg body weight per day. When administered inside the eye or adjacent to the eye, the total dosage per day per eye should be within the range from 1 μg to 1000 μg, the higher dosages being of particular relevance when means of inducing protracted release of active compound are applied.

Supplementary systemic administration of vitamin A and/or local administration of vitamin A or its related compounds to the surface of the eye (topical instillation of eyedrops into the conjunctival sac) may be of value in reducing side-effects of the therapeutic agent owing to its escape from the inside of the eye.

Formulations

The formulation may be a water solution. Alternatively, the formulation may comprise a slow-release formulation or device where the active agent is held confined by mechanical or physico-chemical effects, including polymer binding, co-polymerization, embedding of the active compound in polymers, gels, solids and other substances, adsorption and other types of non-covalent binding. Also included is covalent binding that confers inactivity or sequestration in the bound form together with mechanisms of gradual release that increase or otherwise alter the pharmacokinetic profile of the active agent. Examples of such sustained release systems include semi-permeable polymer matrices in the form of shaped articles (e.g., films or microcapsules). Furthermore, various slow release polymeric devices have been devel-

oped and tested in vivo in recent years for the controlled delivery of drugs. A variety of biocompatible polymers (including hydrogels), including both biodegradable and non-degradable polymers, can be used to form an implant for the sustained release of an invention composition at a particular target site. The generation of such implants is generally known in the art. The present invention is however not confined to the above-mentioned formulations and the skilled person will appreciate that other formulations for effective administration is contemplated by the invention.

The pharmaceutically active compounds may be crystallized as a salt or salts using any counterion that confers to the salt a solubility that is sufficiently rapid or sufficiently slow to provide a desired pharmacokinetic and/or pharmacodynamic profile, as can be determined using conventional kinetics of dissolution assays, microscopic visualization of crystals in the vitreous of the eye, preferably using cross-polarization examination, and dark adaptometric or spectrophotometric examination of the eye.

The pharmaceutically active compounds may be attached covalently to a delivery-enhancing transporter by chemical or recombinant methods and referred to as pro-drugs in that the release (e.g., by degradation or specific cleavage) of the delivery-enhancing transporters from the drugs results in the conversion of the drug from an inactive to an active form. For example, the pro-drug may be produced by esterification, e.g. as a di-X-acetonide, where X is a retinoid. Furthermore, the drug or pro-drug, or a combination of the two, may be crystallized and administered in pure microcrystalline form, in a mixture of crystals with a combination of sizes and coatings that convey a desired and predetermined pharmacokinetic profile and/or susceptibility to disruption by photocoagulation or photodisruptive lasers that may confer lack of drug release or drug release at only low rates before disruption, whereas after noninvasive disruption an increased rate of release from the inactive solid form into water solution inside the eye can be achieved.

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Claims

1. Use of at least one compound capable of inhibiting the visual cycle and/or dark adaptation in an individual in the manufacture of a medicament for prevention or treatment of a non-degenerative retinal disorder, or associated symptoms and complications thereof, in a mammalian.

2. Use according to claim 1, wherein said mammalian is a human.

3. Use according to any of claims 1 and 2, wherein said mammalian is diagnosed with diabetes.

4. Use according to any of claims 1 to 3, wherein the non-degenerative retinal disorder is diabetic retinopathy.

5. Use according to any of claims 1 to 3, wherein the non-degenerative retinal disorder is a disorder associated with diabetic retinopathy.

6. Use according to claim 5, wherein the non-degenerative retinal disorder is macular edema, angioproliferation, or neovascularization.

7. Use according to any of claims 1 to 6, wherein the at least one compound capable of inhibiting the visual cycle and/or dark adaptation comprises at least one retinoid effective of inhibiting the visual cycle in an individual.

8. Use according to claim 7, wherein the at least one retinoid is selected from the group consisting of isotretinoin (13-*cis*-retinoic acid), 11-*cis*-retinol, 11-*cis*-retinal, 11-*cis*-retinyl bromoacetate, -acitretin, adapalene, bexarotene, etretinate, fenretinide, 4-oxo-isotretinoin, tazarotene, motretinid, arotinoid ethyl ester, arotinoid-free carboxylic acid, arotinoid ethyl sulfone, retinaldehyde, *all-trans*-retinyl bromoacetate, *all-trans*-retinyl chloroacetate, and retinoyl betaglucoronide.

9. Use according to claim 8, wherein the at least one retinoid is selected from the group consisting of isotretinoin (13-*cis*-retinoic acid), fenretinide, and etretinate.

10. Use according to any of claims 1 to 9, wherein the at least one compound is composed as a pro-drug.

11. Use according to any of claims 7 to 10, wherein the at least one retinoid is composed as a pro-retinoid.

12. Use according to any of claims 1 to 11, wherein the medicament is in a form for being administered locally.

13. Use according to claim 12, wherein the medicament is in a form for being administered intravitreally.

14. Use according to any of claims 1 to 13, wherein the medicament is in device formulation held confined by mechanical or physico-chemical effects.

15. Use according to any of claims 1 to 14, wherein the medicament is in a slow-release formulation.

16. A method for prevention and/or treatment of a non-degenerative retinal disorder, or associated symptoms and complications thereof, in a mammalian, comprising administering to said mammalian a pharmaceutically efficient amount of at least one compound capable of inhibiting the visual cycle and/or dark adaptation in an individual.

17. The method according to claim 16, wherein said mammalian is a human.

18. The method according to any of claims 16 and 17, wherein said mammalian is diagnosed with diabetes.

19. The method according to any of claims 16 to 18, wherein the non-degenerative retinal disorder is diabetic retinopathy.

20. The method according to any of claims 16 to 19, wherein the non-degenerative retinal disorder is a disorder associated with diabetic retinopathy.

21. The method according to claim 20, wherein the non-degenerative retinal disorder is macular edema, angioproliferation, or neovascularization.

22. The method according to any of claims 16 to 21, wherein the at least one compound capable of inhibiting the visual cycle and/or dark adaptation comprises at least one retinoid effective of inhibiting the visual cycle in an individual.

23. The method according to claim 22, wherein the at least one retinoid is selected from the group consisting of isotretinoin (13-*cis*-retinoic acid), 11-*cis*-retinol, 11-*cis*-retinal, 11-*cis*-retinyl bromoacetate, -acitretin, adapalene, bexarotene, etretinate, fenretinide, 4-oxo-isotretinoin, tazarotene, motretinid, arotinoid ethyl ester, arotinoid-free carboxylic acid, arotinoid ethyl sulfone, retinaldehyde, *all-trans*-retinyl bromoacetate, *all-trans*-retinyl chloroacetate, and retinoyl betaglucoronide.

24. The method according to claim 23, wherein the at least one retinoid is selected from the group consisting of isotretinoin (13-*cis*-retinoic acid), fenretinide and etretinate.

25. The method accoding to any of claims 16 to 24, wherein the at least one compound is composed as a pro-drug.

26. The method according to any of claims 22 to 25, wherein the at least one retinoid is composed as a pro-retinoid.

27. The method according to any of claims 16 to 26, wherein the pharmaceutically efficient amount of said at least one compound is an amount sufficient to inhibit the visual cycle and/or dark adaptation of the treated individual.

28. The method according to claim 27, wherein the pharmaceutically efficient amount of said at least one compound is determined by measuring the level of reduction of dark adaptation in a treated subject.

29. The method according to any of claims 16 to 28, wherein the at least one compound is in a formulation for being administered locally.

30. The method according to claim 29, wherein the at least one compound is in a form for being administered intravitreally.

31. The method according to any of claims 16 to 30, wherein the at least one compound is in device formulation held confined by mechanical or physico-chemical effects.

32. The method according to any of claims 16 to 31, wherein the at least one compound is in a slow-release formulation.

33. A pharmaceutical composition suitable for intravitreal implantation comprising a pharmaceutically efficient amount of at least one compound capable of inhibiting the visual cycle and/or dark adaptation and/or a pharmaceutically efficient amount of at least one retinoid.

34. The pharmaceutical composition of claim 33, wherein said at least one retinoid comprises a retinoid effective of inhibiting the visual cycle in an individual.

35. The pharmaceutical composition of any of claims 33 to 34, wherein said pharmaceutically efficient amount of said at least one compound and/or said pharmaceutically efficient amount of said at least one retinoid is determined by measuring the level of reduction of dark adaptation in a treated subject.

36. The pharmaceutical composition of any of claims 33 to 35, wherein said pharmaceutical composition is in device formulation held confined by physico-chemical effects.

37. The pharmaceutical composition of any of claims 33 to 36, wherein said pharmaceutical composition is in a slow release formulation.

Fig. 1

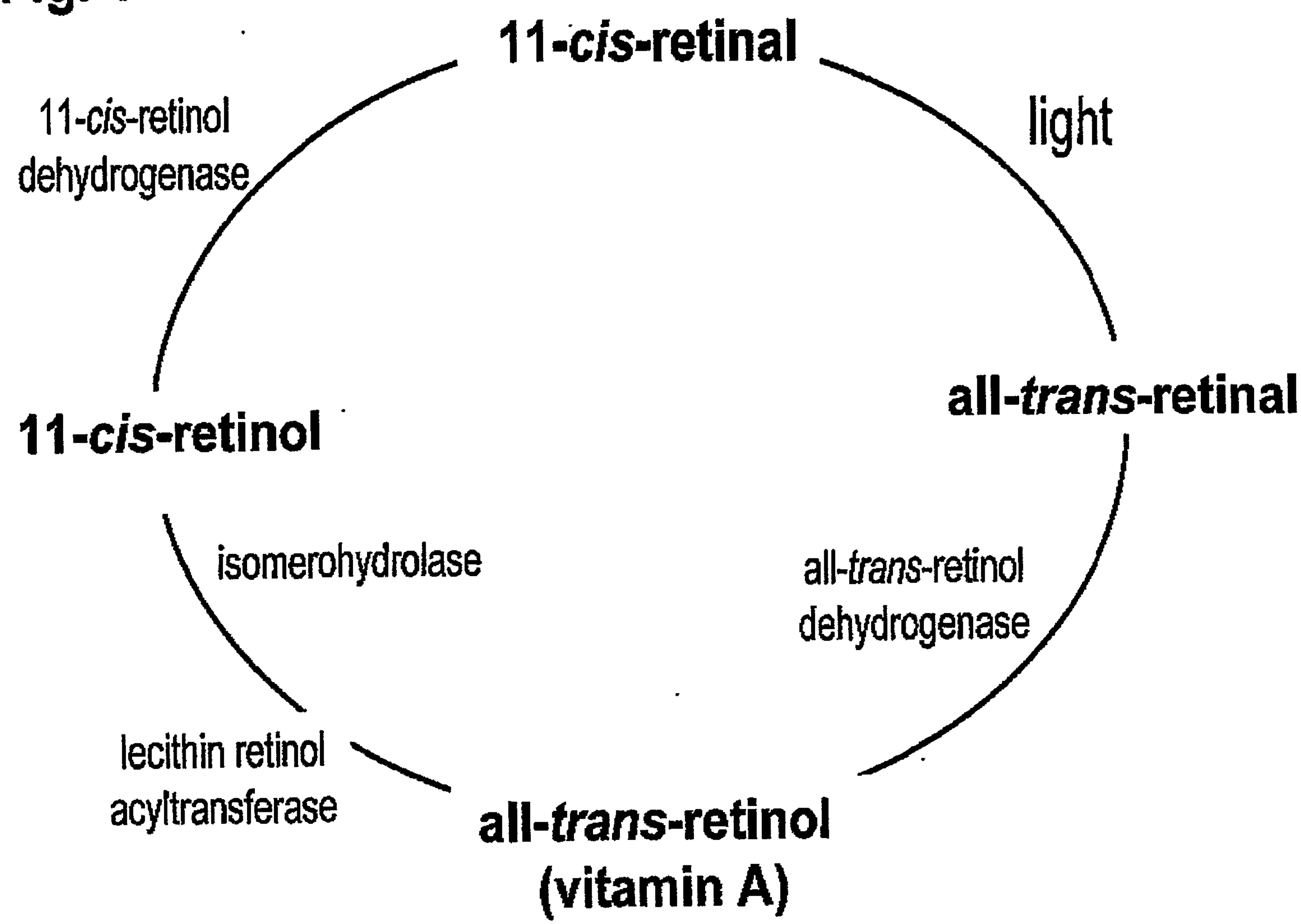
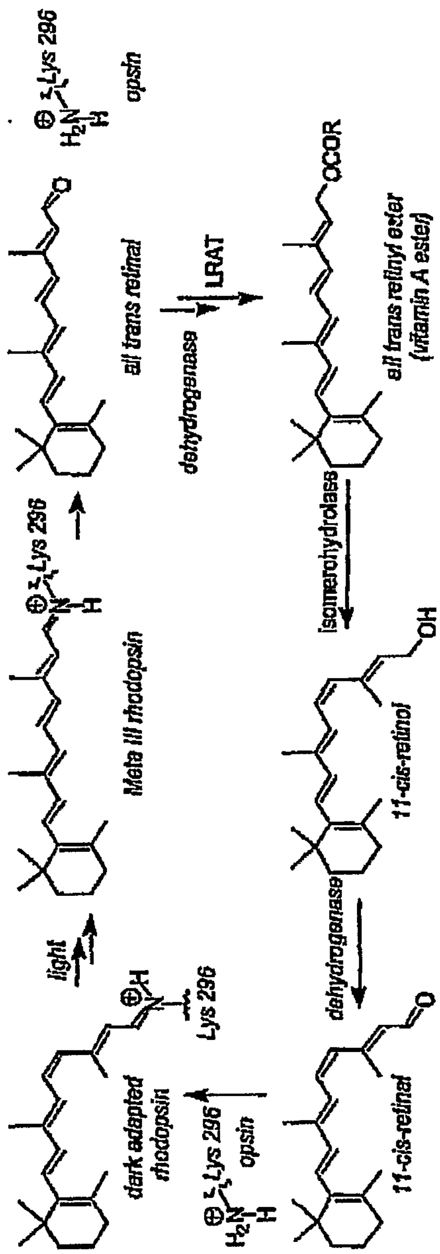
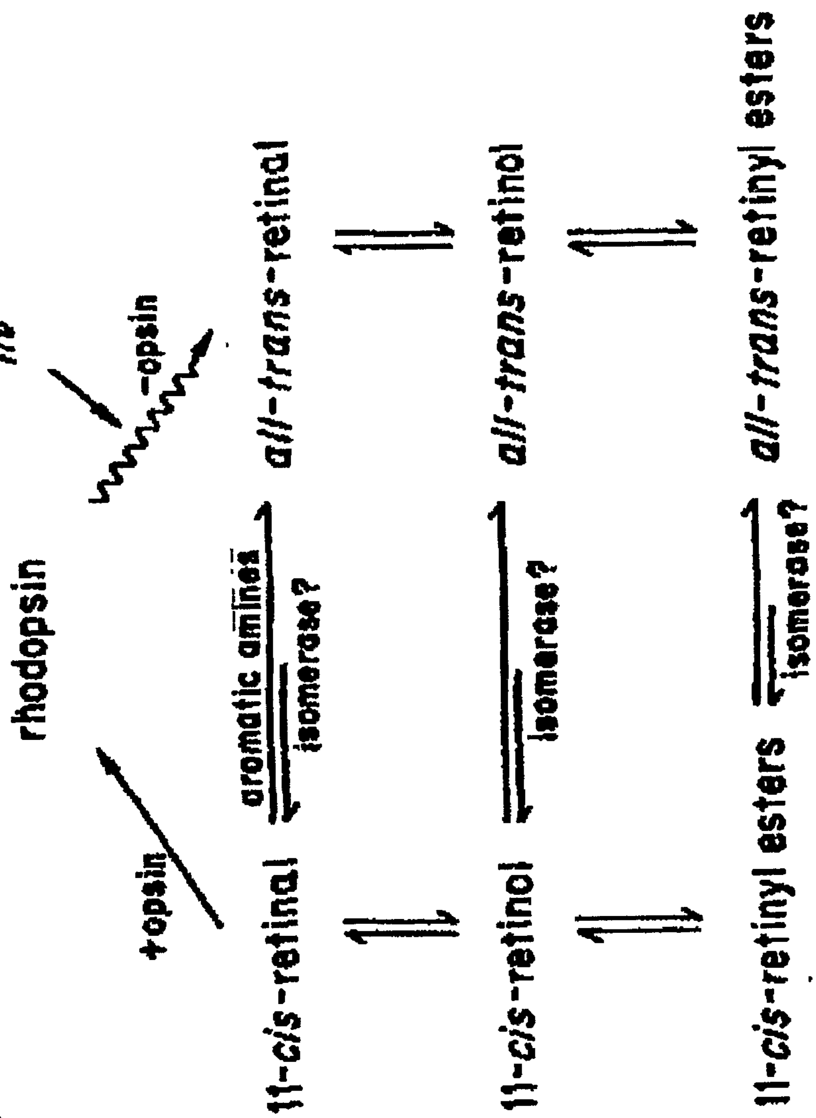

11-*cis*-retinal
light
all-*trans*-retinal
all-*trans*-retinol dehydrogenase
all-*trans*-retinol
(vitamin A)
lecithin retinol acyltransferase
isomerohydrolase
11-*cis*-retinol
11-*cis*-retinol dehydrogenase

Fig. 2



dark adapted rhodopsin
Lys 296
light
Meta III rhodopsin
Lys 296
all trans retinal
Lys 296
opsin
dehydrogenase
LRAT
all trans retinyl ester (vitamin A ester)
OCOR
isomerohydrolase
11-cis-retinol
OH
dehydrogenase
11-cis-retinal
Lys 296
opsin

Fig. 3


rhodopsin
hν
-opsin
+opsin
11-cis-retinal
all-trans-retinal
aromatic amines
isomerase?
11-cis-retinol
all-trans-retinol
isomerase?
11-cis-retinyl esters
all-trans-retinyl esters
isomerase?